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Enset (Ensete ventricosum (Welw.) and Its Biotechnology with Special Focus on Ethiopian Condition

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Abstract
Due to the long regeneration time and seed recalcitrance to germination, improvement of enset through conventional plant breeding appears to be impractical. Improvement of traits in important enset clones could be achieved through modern biotechnological technique such as genetic transformation. Hence development of transformation procedures for the improvement of these agronomically desirable traits in enset is essential. The application of molecular markers has enabled studies on reproduction, speciation and spatial-temporal dynamics of populations in many species. DNA-based markers play an important role in estimating diversity, identification of genotypes and tagging genes with desirable traits. Random amplified polymorphic DNA (RAPD) is one of the methods to estimate the genetic diversity and relatedness in cultivated enset.
Keywords: Enset, Biotechnology, Molecular marker, Micopropagation, Genetic transformation

1. INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Chessman is a perennial, herbaceous and monocotyledonous crop in the family Musaceae and strongly resembles banana morphologically (Westphal 1975). The corm and pseudostem of enset yield a highly carbohydrate-rich staple or co-staple food, for the quarter of the Ethiopian population that inhabits the south and south-western part of Ethiopia. Enset is a crop that tolerates prolonged drought periods, flooding and many diseases. Due to its drought tolerance, it is regarded as a priority crop in Ethiopia, where it makes a major contribution to the food security of the country. Regions where enset is used as staple food are usually less affected by the recurrent drought periods that occur in Ethiopia (Brandt *et al.* 1997). Enset is suitable for sustainable agricultural systems due to its contribution to soil fertility. In addition, enset can easily be stored without the need for refrigeration and is available throughout the whole year. It can be accessed at any time when there is food shortage and other crops fail as a result of drought, diseases or other factors. However, enset has been harvested so intensively during droughts that some important clones have become extinct, thereby reducing the genetic diversity of the crop.

Enset represents 65% of the total crop production in the southern regions of Ethiopia. Productivity is very high compared to other crops but varies depending on edaphic factors, altitude, cultural practices and varietal differences. Although estimating the productivity of enset has proved difficult, Bezunhe (1996) reports that enset plants grown 5 metres apart, or at 2000 plants/hectare, yield between 18.5 and 29.8 kg/plant or between 7,414 and 11,950 kg/ha/year. An integrated and comprehensive study of the biological, agricultural, ecological, social, and economic components that make up enset based agricultural systems is needed in order to boost productivity and permit the distribution of enset products to non-enset growing regions of Ethiopia.

2. THE ENSET CROP--- ENSETE VENTRICOSUM

An enset plant has three main parts, an underground corm, an aerial pseudostem made of overlapping leaf sheaths, and several broad leaves. Enset is a large plant with a height of 12 m and a pseudostem that is dilated at the base and that has a width of up to 1 m. The growing point of the plant or meristem is located at the junction between the pseudostem and the underground corm near the soil surface. When the plant matures after 9–14 years, the true stem emerges through the leaf sheaths and produces inflorescences. The best time for harvesting is just after production of inflorescences and seed set. However, enset sometimes can be harvested when only 3-4 years-old, depending on the clone and growing conditions. If not harvested, the whole plant falls down, shortly after seed set (Taye, 1996).

2.1. Uses of Enset Crop

Enset is a multipurpose crop with all plant parts being utilized for human food, animal forage, medicinal or ornamental uses. Enset has high significance in day-today-life of the peasant households cultivating this crop as staple food. The peasants indicate that enset is their food, their cloth, their house, their bed, their cattle-feed and their plate (Brandt *et al*. 1997). Amongst its uses, enset produces a starchy, carbohydrate-rich food for human consumption. This is prepared in different ways by the ethnic groups that use enset as staple or co-staple food. However, three dishes are most common. A steam-baked flat-bread ‘kocho’ is prepared from the pulp of fermented pseudostem and corm. ‘Bulla’ is made from solidified starch obtained from the pseudostem and is used as porridge or as a flat-bread, while as ‘amicho’ where the corm is eaten boiled like potato. Nowadays,

enset flour is also mixed with flour from cereal crops such as tef (*Eragrostis tef*) to make the traditional Ethiopian bread 'injera'. Although rich in carbohydrate and starch, enset foods are low in protein and vitamin A (Taye, 1996).

The fibre from the leaf and pseudostem is comparable to that of Musa textiles and is used for construction and rope-making. The leaves are used as animal forage and for wrapping. The corm and pseudostem of some enset clones are used as traditional medicine to repair broken bones, facilitate abortions or discharge placentas after birth in both cattle and humans, and possess antimicrobial properties (Hölscher & Schneider 1998). Enset is an attractive evergreen ornamental which is being introduced to different geographical regions of Ethiopia and other neighbouring countries. The presence of 20–40 magnificent huge enset plants framing small huts and farmyards presents an impressive picture (Shank 1994).

Enset is a multipurpose crop of which every part is thoroughly utilized. It is a good source of starch. The corm and the pseudostem are the most important sources of food. The types of food from these parts are known as 'Kocho', 'Bulla' and 'Amicho' (Spring *et al.,* 1996). Kocho is the bulk of the fermented starch obtained from the decorticated (scraped) leafsheathes and grated corm. Bulla is obtained by squeezing out the liquid containing starch from scraped leafsheathes and grated corm and allowing the resultant starch to concentrate into white powder. Amicho is boiled corm of young enset plants known for best quality of corm. It is prepared and consumed in a similar manner to preparation of other root and tuber crops (Brandt *et al.,* 1997). Fiber is the by-product of enset that is left after decorticating the leaf sheathes. Its strength is found to be equivalent to the important fiber crop *Musa texstalis* (abaca) (Taye, 1996). Fiber is used for making bags, ropes, twines, cordage, mats, etc where the variety, the age of the plant, and the way in which the fiber is extracted and stored determine its length and quality.

Enset is one of the major crops that can significantly help to ensure food security in a country like Ethiopia. The average yield of refined enset product kocho ranged from 7 to 12 tons/ha/ year. The amount of food attainable from 50-60 enset plants per year could provide enough food for an average family of 5-6 persons (Zeweldu and Ludders, 1996). Enset products are available throughout the year and can be stored in pits for long periods of time without spoiling.

As a result of its many uses, enset cultivation is deeply entwined in the economic, cultural and social life of different ethnic groups. Early travelers in the Wolayta region of southern Ethiopia called the human population and the region 'the enset culture' or 'the enset people' impressed with the importance apparently attached to this crop (Shank 1994).

2.2. Similarity and Differences of Enset and Banana

In addition to other synonyms, enset is often known as false banana due to its physical resemblance to a banana plant. However, enset belongs to the genus *Ensete* in the banana family Musaceae while banana belongs to the genus *Musa*. Although enset produces banana-like fruits these fruits do not constitute the edible part, which is instead found in the underground corm and in the aerial pseudostem made up of overlapping leaf sheaths. Starchy food is produced by pulverizing the corm or scraping the pseudostem, followed by a short fermentation period. Unlike banana, enset is monocarpic and fruits only once in its life cycle. The fruits contain several seeds which are hard and about 1-2 cm long. Sprouting occurs only when the main shoot with the meristem, is artificially decapitated at the junction between the pseudostem and corm at soil surface, while in banana sprouting occurs spontaneously. Enset is a diploid plant with the haploid chromosome number n = 9, whereas *Musa* species, including edible banana, have different ploidy levels and chromosome numbers (diploid, triploid or tetraploid), with n = 10 or n = 11 (Ude *et al.* 2002)**.**

2.3. Distribution of Cultivated and Wild Enset

Of the six commonly recognized species of *Ensete*, *E. superbum* and *E. glaucum* grow wild in Asia, *E. perrieri* in Madagascar and *E. gilletii*, *E. homblei* and *E. ventricosum* in eastern Africa and some species of *Ensete* are also reported to grow in North America (Simmonds 1962). *Ensete ventricosum* is considered to be the only wild species growing in Ethiopia (Simmons 1956). *Ensete ventricosum* was previously cultivated only in the south and south-western parts of Ethiopia, but the recurrent droughts have led to the expansion of enset cultivation to other parts of the country. A wide adaptation within the species to altitude, soil and climate has allowed widespread cultivation in western Bale, south-western Oromia including south and east Shewa, Jima, Illubabor and Welega (Shank 1994). Wild enset grows at altitudes of 1200–1600 m above sea level while domesticated enset is cultivated at altitudes of 1100–3100 m above sea level (Brandt *et al.* 1997). The optimal conditions for enset cultivation occur at 2000–2750 m with 1100–1500 mm rainfall, a temperature range of 10–21 ºC and a relative humidity of 63–80% (Brandt *et al.* 1997). Lack of sufficiently high humidity is more limiting for good growth than high temperatures. Enset often grows best in acidic, heavy clay soils that retain high levels of organic matter when manured (Shank 1994).

2.4. Research Activities on Enset

There is no enough research has been conducted on the genetic improvement of enset. One reason is the long regeneration time, which ranges from **9 to 14** years. The recalcitrant nature of enset seeds to germination may also have contributed to the lack of work using conventional plant breeding techniques. Due to these and other reason, most of the research on enset has been concentrated on agronomic studies of e.g. yield and productivity, plant density and intercropping (Bezunhe, 1996).

Enset germplasm collection and *ex situ* conservation have been conducted by the Areka Research Station, where 77 and 128 enset accessions from Wolayta and Kembata administrative regions, respectively, is now grown *ex situ*. For *ex situ* conservation, back-up samples *in vitro* would, however, be very useful in order to avoid loss of accessions due to biotic and abiotic factors. Characterization of the germplasm of domesticated enset has been conducted using morphological traits (Tsegaye & Struik 2002). For a general estimate of genetic variability, modern molecular marker techniques are often preferred. Recently, an Amplified Fragment Length Polymorphism (AFLP) study was conducted to evaluate the germplasm of cultivated enset in some enset-growing regions of Ethiopia (Negash *et al.* 2002).

2.5. Problems Associated with Enset Cultivation

Although enset is thought to be resistant to many biotic and abiotic potential problems, the crop is now threatened by some destructive diseases such as bacterial wilt caused by *Xanthomonas campestris* pathovar (pv) *musacearum* and fungal diseases caused by *Mycosphaerella musicola* and *Sclerotium rolfsi* (Quimio & Tessera 1996). Quimio and Tessera (1996) indicated the absence of effective control measures for the diseases, pests and mole rats that affect enset cultivation and that may cause the loss of some important clones. Bacterial wilt is a very destructive epidemic disease of enset and banana often reported in some east African countries. Hence farmers must be informed about measures that can be taken in order to counteract the mechanical transmission of this disease. In addition, the presence of resistance in enset clones needs more investigation. Research on the genetic diversity and distribution of *Xanthomonas campestris* pv *musacearum* in Ethiopia could also be a useful approach for the development of effective control measures. More pathogenicity tests are needed on microbes isolated from enset or reported as pathogens in other crops such as *Ralstonia olanacearum* (Quimio & Tessera, 1996).

Although enset foods are known to have high starch content, they are highly deficient in proteins and vitamin A. This has caused some malnutrition-related diseases in regions where the crop is used as the staple food and hence the introduction of other crops rich in vitamins and proteins to the daily diet is essential in order to improve the health status of these people, in particular, small children. Development of enset clones with disease resistance, highly productive and enriched vitaminsand/or proteins through biotechnological techniques (tissue culture, genetic transformation and molecular marker) and dissemination of the clones to these regions could be of great value.

3. TISSUE CULTURE TECHNIQUES

Plant tissue culture techniques involve the growing and multiplication of totipotent cells, tissues and organs of plants on defined solid or liquid media comprising nutrients under an aseptic and controlled environment (George 1993). *In vitro* techniques are used for cryopreservation, conservation of rare and highly endangered plants and production of secondary metabolites. Furthermore, *in vitro* regeneration and micropropagation methods are now widely used for the improvement of important crops through biotechnological methods such as genetic transformation. Development of improved crops through genetic transformation can only be achieved if an optimum procedure for regeneration of transformed explants is available.

Micropropagation is one of the tissue culture techniques used for the production of ‘disease-free’, high quality and uniform planting material. It enables production of large numbers of propagules within a relatively short time for commercial purposes, independent of season. However, the full application of these techniques depends on the cost of micropropagation. Micropropagation could be achieved either using axillary shoots, shoot buds or nodal segments, or from adventitious shoots using parts of leaves, petals, flowers or roots. Adventitious shoots can be obtained directly or indirectly through a callus phase. Micropropagation directly from axillary buds is more desirable for routine micropropagation, since it produces true to type propagules. Indirect regeneration through callus might be more suitable for genetic transformation, due to the disorganized, highly dividing nature of callus cells and thus the relative easiness to transfer DNA. Furthermore, regeneration of shoots from transformed callus usually results in complete transformed plants. By contrast, direct regeneration might result in the production of chimeric transgenic plants. However many crops, in particular monocots, are recalcitrant to the induction of callus and hence in such cases direct regeneration may be necessary. Furthermore, micropropagation of some crops is hindered by the recalcitrant nature of certain tissues, slow growth and loss of cultures as a result of endophytic microbe invasion and exudation of growth-inhibiting substances such as phenols (Zeweldu & Lüdders 1998). In order to overcome these problems, preparatory or preventative measures

need to be devised in parallel to the micropropagation procedure. Diagnosis for endophytic microorganisms in the preparatory stage is important in order to prevent loss of cultures at a later stage and to ensure the production of 'disease free' plants.

3.1. Micropropagation of Enset Clones

Cultivated enset is generally propagated by production of suckers due to the long generation time during sexual reproduction and the recalcitrant nature of seeds to germination. Micropropagation could be highly useful for enset but has unfortunately proved to be exceptionally difficult due to phenol exudation, internal contamination and slow growth (Zeweldu & Lüdders 1998). In order to minimized the problem of phenol oxidation there is a need of adding activated charcoal to the medium. Prior to shoot multiplication, the cultures were screened for some common bacterial and viral diseases of enset and banana, as they often are cultivated in adjacent fields and are attacked by common pathogens. *Ralstonia solanacearum*, wilt causing bacterium, was identified from one enset clone using ELISA and this clone was discarded from further micropropagation. This bacterium was previously reported to be a pathogen of banana and was found in enset (Prior and Steva 1990), but no pathogenicity test was conducted.

In spite of the difficulty involved in enset culture, development of an efficient micropropagation system for enset clones through meristem wounding, and modification of the MS (Murashige & Skoog 1962) nutrients according to the culture stage. The medium components, in particular the MS macronutrients, were modified based on the results of nutrient analysis of glasshouse-grown leaves from enset sprouts (Genet, 2004). In addition, three developmental stages were defined that enabled the production of 75 shoots from a single wounded explant in one subculture. The three main steps were initiation, bud proliferation and shoot and root elongation. Furthermore, wounding the meristem was found to be essential in order to obtain highly proliferating shoots and unwounded explants produced only one or, in very rare cases, two shoots. As Genet (2004) reported, out of the four different media compared, two media which contained modified MS macronutrients with single or double strength micronutrients (EV-2MS), produced a higher number of propagules. Earlier, a procedure for micropropagation of enset has been reported by Negash *et al.* (2002), but only 2-3 shoots were produced from a single explant. Zeweldu & Lüdders (1998) reported their attempts to obtain shoot multiplication failed in unknown species of *Ensete* due to high oxidation of phenols which resulted in the death of explants. According to Genet (2004) the procedure in the study involved direct regeneration from swollen corm with no intervening callus phase securing true-to-type propagules.

4. GENETIC TRANSFORMATION

Genetic transformation techniques, unlike conventional breeding, have more applicability in the improvement of crops with a long regeneration time. Development of crops with agronomically desirable traits through genetic engineering can be achieved directly through particle bombardment or electroporation or indirectly through *Agrobacterium*-mediated transformation. *Agrobacterium*-mediated transformation is the preferred method since only one or few copies of the transferred DNA (T-DNA) harbouring the foreign gene is transferred. Moreover, rearrangement of the introduced DNA occurs rarely in *Agrobacterium*-mediated transformation compared to direct gene transfer methods. Transformation of monocot plants through *Agrobacterium* has been thought to be unachievable due to the putative inability of *Agrobacterium* to infect monocotyledonous plants (Kaul et al., 2004). However, the ability of the bacterium to infect and transmit DNA has been demonstrated in many major monocot crops such as rice, barley and wheat (Cheng *et al.,* 1997).

Agrobacterium transformation and transfer of the T-DNA to the plant cell is a complicated process. This process is triggered by activation of a series of virulent (*vir*) genes residing on the tumour inducing (Ti) plasmid of the bacterium, through signals from the host plant cell. The activation of *vir* genes results in the generation of site-specific nicks within the T-DNA borders and production of linear single-stranded DNA molecules in the plant cell. The T-DNA is encoded in the Ti-plasmid and is defined by 25-bp conserved border sequences at both left and right ends. These border sequences are the only cis-or-trans acting elements needed for DNA transfer. Genes essential for DNA transfer are encoded within the *vir* region of the Ti-plasmid. Transcription of *vir* genes is regulated by *virA* and *virG* genes. An increase in the copy number of *virG* on a multicopy plasmid leads to an increase in *vir* gene expression which might in turn enhance the efficiency of DNA transfer in plants (Han *et al.,* 1997). Once phosphorylated by *virA*, *virG* transcriptionally activates the other vir genes. Plants produce different compounds which result in different levels of gene expression in different hosts, thereby affecting their sensitivity to infection by *A. tumefaciens*. A low level of *vir* gene expression caused by low inducing ability of the host plant reduces the potential of the bacterium to infect the host plants (Han *et al.,* 1997).

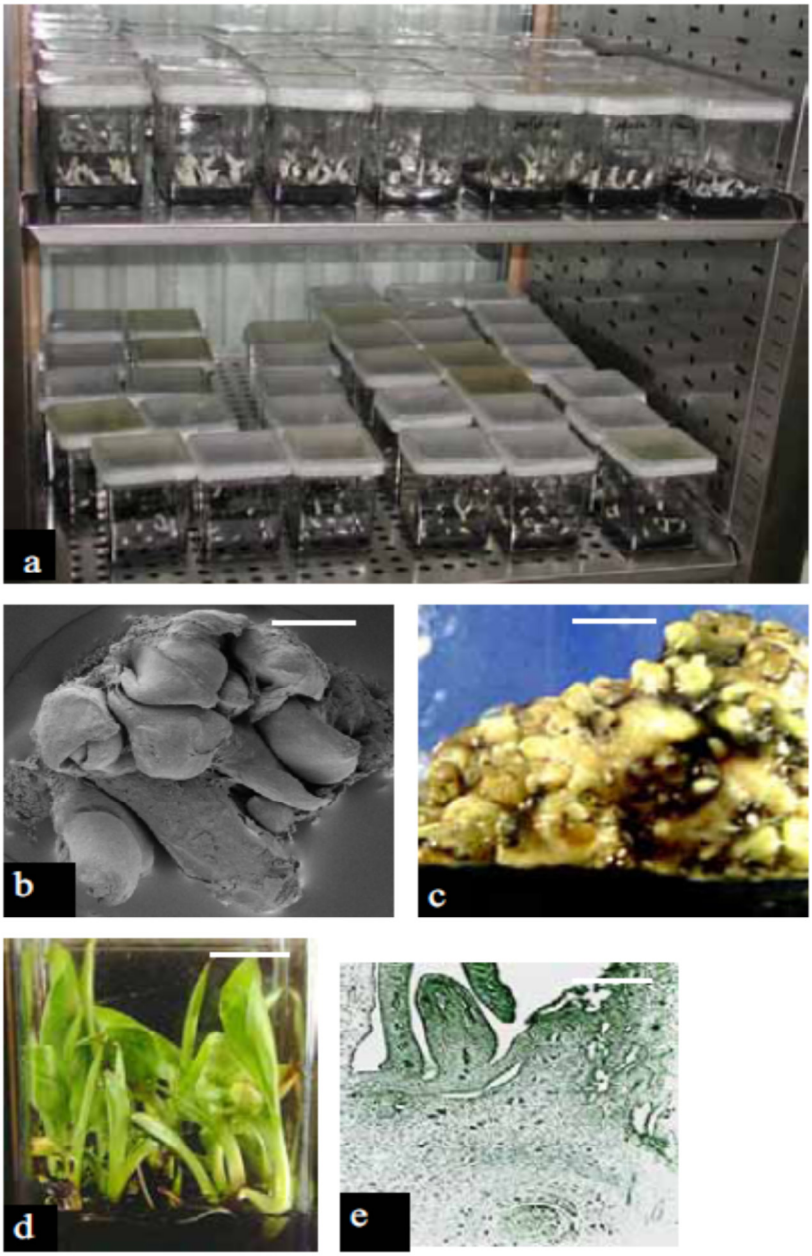

Figure1. a) Initiation of enset cultures in growth cabinet, b) scanning electron micrograph of proliferating shoots on a corm piece, c) formation of multiple buds on swollen corm d) shoot elongation and rooting, e) shoot meristem originating from the internal part of a corm piece. Bars represent 1 cm in d and 0.3 mm in b, c and e. Source (Genet, 2004)

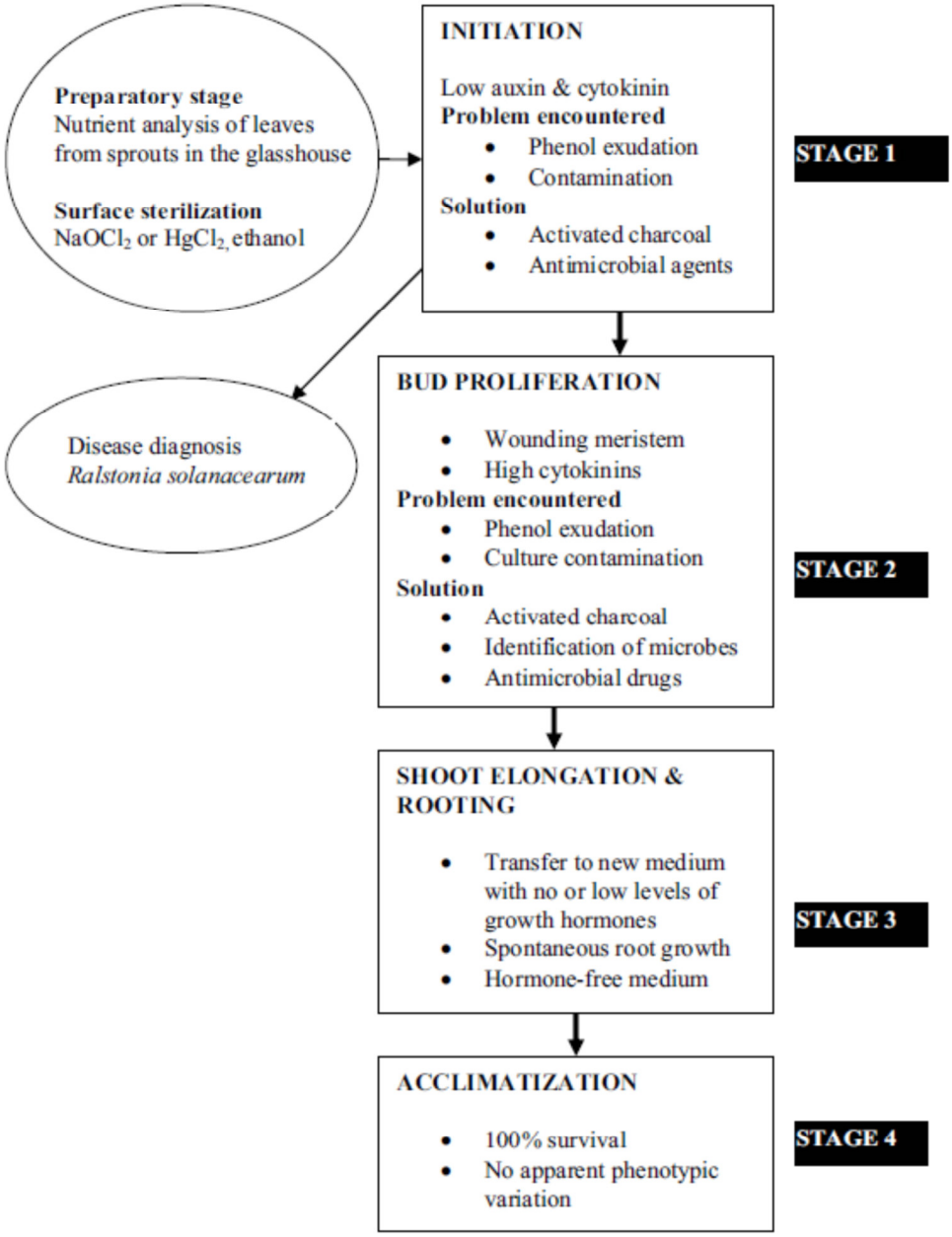

Figure 2. The major steps and procedures followed in micropropagation of enset clones. Source (Genet, 2004)

4.1. Genetic Transformation of Enset

Enset has a very long regeneration time which ranges between 9 and 14 years depending on the clone and altitude at which it is grown. Due to the long regeneration time and seed recalcitrance to germination, improvement of enset through conventional plant breeding appears to be impractical. Although enset is known to have desirable traits such as high productivity, it has some drawbacks that require improvement to maintain the sustainability of the crop. For instance, enset is a simple basic starch crop, quite low in protein, and vitamin A, and is attacked by some destructive diseases such as bacterial wilt and fungal diseases like *Mycosphaerella musicola* and *Sclerotium rolfisi* (Quimio & Tessera 1996). Improvement of these traits in important enset clones could be achieved through modern biotechnological technique such as genetic transformation. Hence development of transformation procedures for the improvement of these agronomically desirable traits in enset is essential.

Genet (2004) developed a transformation procedure for enset through both *Agrobacterium* and particle bombardment techniques as confirmed by **gus** gene expression. Two plasmid constructs were used for particle bombardment. The first plasmid, pC3301-1, carried the antifungal genes 1,3-glucanase, chitinase, the reporter gene, glucuronidase (*gus*), the selectable marker gene phosphinothricin-N-acetyltransferase (*bar*). The second plasmid, pC3301-2, carried all the above genes except the chitinase gene. For *Agrobacterium*-mediated

transformation, the *Agrobacterium tumefaciens* strain EHA 105 harbouring pC3301-2 was used. All explants types used such as shoot tips, leaves, roots and zygotic embryos showed *gus* expression indicating that enset although a monocot, can be infected with *Agrobacterium.* The different explants expressing *GUS* expression after *Agrobacterium* infection and particle bombardment are shown. In this procedure, the particle bombardment involved sonication of explants for 100 s, plasmolysis of tissues with 0.25 M sorbitol and mannitol each, for 2 hrs prior to transformation, and co-cultivation of infected tissues in the presence of acetosyringone (virulence inducing compound) (Genet, 2004. The bombardment conditions were 3 cm target distance, 1100 psi rupture disc pressure and vacuum level at 25 inches Hg. *Agrobacterium*-mediated transformation showed a higher percentage of GUS positive shoot tip (63%) and leaf (50%) explants in clone Erba, when the explants were sonicated prior to transformation (Genet, 2004). Particle bombardment gave a higher percentage of GUS expressing explants when sonicated leaves (60%) of clone Erba and shoot tips (49%) of clone Feresae were bombarded (Genet, 2004). Four days of co-cultivation was found to be optimum to obtain a higher percentage of GUS expressing explants with lower loss of cultures due to bacterial overgrowth as a result of long exposure time. In this experiment, shoot tips and leaf explants were the best tissues in both transformation techniques. In particle bombardment, pC3301-2, which is the smaller plasmid, showed a higher percentage of GUS expressing explants than pC3301-1(Genet, 2004). As Genet (2004) indicates that, the expression of *gus* gene is affected by sonication treatment, plasmid size, co-cultivation period and transformation methods.

5. APPLICATION OF MOLECULAR MARKER ON GENETIC DIVERSITY

Molecular markers are molecules that could be used to trace a desired gene(s) in examined genotypes. In fact a piece of DNA or a protein can be used as a marker. Earlier approaches that made selection of specific traits easier were based on the evaluation of morphological traits (Staub *et al*., 1996), isozymes (Stuber & Khanna 1991), storage proteins like glutenins, gliadins, hordeins, etc. (Shariflou *et al.,* 2001). However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective.

To test genetic resources for their productivity, quality parameters and stress tolerances field trials and chemical/physical/biochemical tests are employed. Field trials are usually time consuming, therefore molecular markers and DNA technology are used to assess diversity in the gene pool, to identify genes of interest and to develop a set of markers for the screening of progenies (Karp *et a.,* 1998).

Human beings in their struggle for survival have selected and domesticated crops to fulfill their immediate needs. Nowadays, many people have become aware of the importance of preserving genetic diversity for the survival and continuation of any form of life that exists on earth. Data on the relative genetic diversity within and among plant populations can have a major significance in the preservation of genetic diversity in crops and the improvement and maintenance of crop germplasm for sustainable agriculture. Several modern molecular techniques are now being applied together with morphological studies to investigate genetic diversity and relatedness in crops. The application of molecular markers has enabled studies on reproduction, speciation and spatial-temporal dynamics of populations in many species (Xu *et al.* 2002). Multilocus fingerprinting techniques, such as Random amplified polymorphic DNA (RAPD**)** provide a cost-effective means to rapidly and simultaneously assess the genetic variability across many loci (Call *et al.,* 1998). Use of a marker system such as RAPD, which has a relatively low cost, will make its wider-application more feasible. The questions on homology of RAPD bands and their reproducibility are now being answered. Reproducibility can be ensured if the experimental conditions are carefully optimized prior to the full application of the technique. Furthermore, bands of the same size are usually homologous when samples from the same or closely related species are investigated (Rieseberg, 1996). Lannért *et al.* (1996), investigated the homologous nature of RAPDs and showed that out of 250 RAPD-markers recorded, only 1.2% displayed lack of hybridization signal, indicating success in their application.

5.1. Genetic Diversity in Cultivated Enset Clones

DNA-based markers play an important role in estimating diversity, identification of genotypes and tagging genes with desirable traits. Random amplified polymorphic DNA (RAPD) is one of the methods to estimate the genetic diversity and relatedness in cultivated enset. To verify this, 111 cultivated enset samples taken from 9 different geographical sites representing all the major enset-growing regions of Ethiopia (Genet, 2004). A total of 126 primers were tested for reproducibility. Of these 12, were selected for analysis in which they subsequently produced 72 repeatable and polymorphic bands. Each cultivated enset clone is propagated vegetatively, but the genetic diversity among the clones was found to be relatively high compared to that of most other outbreeding crops (Nybom & Bartish 2000). Several other studies have shown that variation among genotypes is comparable regardless of whether they reproduce vegetatively or not (Hamrick & Godt 1996). In this study, all the 111 enset clones investigated exhibited unique banding patterns. The results indicated that the diversity and number of

clones in enset cultivation regions could be as high as the number of vernacular naming used by local farmers. Alemu & Sandford (1996) were reported; more than 160 enset clones with different vernacular names have been recorded from the northern part of Omo River alone. Shank (1994) also noted the presence of considerable variation within the species for characters associated with growth and adaptation. In Genet's (2004) study, in addition to only sampling clones with different names, other factors may also have contributed to the high genetic variation that she observed. Among these factors are somatic variation, maintenance and protection of enset clones due to cultural reasons and perceived agronomic value (quality of food, fibre, medicine etc), introduction of new clones from other regions and occasional gene flow from wild enset possibly from other *Ensete* species too.

The genetic variability recorded for the 9 different sampling sites is higher withinsite diversity in the extreme western and southern parts of the enset growing regions of the country. Partitioning the total variation demonstrated higher variation within the different sites (86%) than among sites (14%) (Genet, 2004). Principal co-ordinate analysis (PCO) showed that the northeastern sites Answae, Seltae and Setunae were rather strongly differentiated from the other six sites. This variation in genetic diversity between sites could be due to climatic reasons, availability of germplasm or cultural history and the extent of dependency on enset as a food source. Genetic variability was not related to geographical distances but rather to the distribution pattern and extent of enset use in the various ethnic groups that cultivate this crop (Genet, 2004).

5.2. Genetic Diversity in Wild Enset

RAPD markers were applied to investigate genetic diversity in wild germplasm in Ethiopia. This study was based on 48 wild enset plants from 5 different sites around the Bonga area, in the south-western part of Ethiopia (Genet, 2004). In addition, 9 representative cultivated clones and 8 *Musa* accessions were also analysed. In the work of Nybom & Bartish (2000), genetic differentiation among the 5 wild enset populations was estimated with Analysis of Molecular Variance (AMOVA) and found to be very low in comparison with other outbreeding, perennial species. In addition, there was no correlation between genetic and geographical distances among the 5 wild populations. This low differentiation could be attributed to the relatively small distribution area of wild enset in Ethiopia and/or the relatively short distance between the sampling sites, with the maximum distance between the sampling sites being 70 km (Genet, 2004). The within-population variation using Jaccard's similarity coefficients ranged from 0.65 to 0.69, with a mean of 0.67, and using the Shannon index from 0.58 to 0.67, with a mean of 0.63 (Genet, 2004). These values were lower than the values obtained for cultivated enset (0.52–0.81 with a mean of 0.74). One reason for this low diversity in wild enset could be due to the low germination ability of enset seeds. Cluster analysis based on unweighted pair group method with artithmetic averaging (UPGMA) and principal component analysis (PCA) demonstrated that the wild enset samples cluster separately from cultivated enset, suggesting that the present-day cultivated enset in Ethiopia originated from very few wild progenitors (Genet, 2004). Gene flow between cultivated and wild enset is probably very restricted. This is because enset has a mixed mode of reproduction in which the wild enset has an outbreeding system and reproduces through seeds, while cultivated enset is generally propagated vegetatively since the plant is harvested for food before seed set. Two populations located in a forest area showed somewhat higher diversity than the remaining three. This result suggests that populations with less human interference may harbor more diversity than populations close to human settlements. Overall, the relatively high total genetic diversity observed in wild enset indicates the presence of a large gene pool which may harbor important genes that can be utilized in the improvement of enset clones. In addition, *Musa* species, as close relative of enset, may also constitute a valuable source of genes with desirable agronomic traits (Genet, 2004).

6. CONCLUSION

Enset is a multipurpose crop with all plant parts being utilized for human food, animal forage, medicinal or ornamental uses. It has high significance in day-today-life of the peasant households cultivating this crop as staple food and it is one of the major crops that can significantly help to ensure food security in a country like Ethiopia. However, there is no enough research has been conducted on the genetic improvement because of the long regeneration time, which ranges from 9 to 14 years. So in order to develop enset clones with disease resistance, highly productive and enriched vitaminsand/or proteins through biotechnological techniques, we can use tissue culture, genetic transformation and molecular marker as a tool of modification. Cultivated enset is generally propagated by production of suckers due to the long generation time during sexual reproduction and the recalcitrant nature of seeds to germination. Micropropagation could be highly useful for enset but has unfortunately proved to be exceptionally difficult due to phenol exudation, internal contamination and slow growth. In order to minimized the problem of phenol oxidation there is a need of adding activated charcoal to the medium. In spite of the difficulty involved in enset culture, development of an efficient micropropagation system for enset clones through meristem wounding, and modification of the MS nutrients according to the culture stage, the medium components, in particular the MS macronutrients, were modified based on the results of nutrient

analysis.

Genetic transformation techniques, unlike conventional breeding, have more applicability in the improvement of enset with a long regeneration time. Development of crops with agronomically desirable traits through genetic engineering can achieved directly through particle bombardment or electroporation or indirectly through *Agrobacterium*-mediated transformation.

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